EXPERIMENTAL STUDY ON THE NUTRITIONAL SIGNIFICANCE OF FAT FROM THE VIEW-POINT OF ITS EFFECTS ON THE LIVER GLYCOGEN CONTENT

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by

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INTRODUCTION

Fat is known to have the greatest caloric value of all the foodstuff. Being both synthesized and stored within the body, fat has been regarded as an optional caloric source; however, advanced studies of the essential fatty acids revealed clearly the fact that fat served not only as a calorigenic material but also had specific physiological significance. Our laboratory has succeeded in preparing a fat emulsion which can be safely given intravenously. Since then, parenteral nutrition with this emulsion has been used for surgical cases with satisfactory result. The mechanisms by which the nutritional effects of fat are produced are still not completely understood. The author has attempted to clarify these obscure mechanisms and to discuss the reality of the so-called harmfulness of fat on liver function, taking particular note of the observations concerning liver glycogen content of rabbits and rats.

I. EFFECTS OF INTRAVENOUS INFUSIONS OF FAT EMULSIONS ON LIVER GLYCOGEN CONTENT AND FATTY LIVER PRODUCTION IN RABBITS

1. Experimental Methods

Experimental Animals: Healthy male rabbits weighing about 2.0～2.5 kg were used. They were fed ad libitum with a diet consisting of bean-curd-waste and green grass during the course of the experiment.

Fat Emulsions: A 20% sesame oil emulsion and 20% cod liver oil emulsion were used. These emulsions were specially prepared for use in the present experiment and do not contain glucose, although the sesame oil emulsion for clinical use (Fatgen) contains glucose in a concentration of 7%. Each of these emulsions was infused daily into the ear vein of rabbits without any supplemental drug or dilution. The daily dose was set as 10.0 cc per kg of body weight which was three times the quantity of the standard dose defined in our laboratory.

Determination of the Liver Glycogen Content: After 24 hours of fasting, the rabbits were laparotomized under Nembutal anesthesia with a small incision of 2 cm in length, and about 5 g of liver tissue was excised; the bleeding from the liver stump was stilled with a mass ligature and the abdominal wall was closed with sutures. About 0.5 g of each excised liver was examined to determine the glycogen content by SOMOGYI and KRAMER's method. Values were described in terms of mg per 100 g of moist weight of liver tissue.

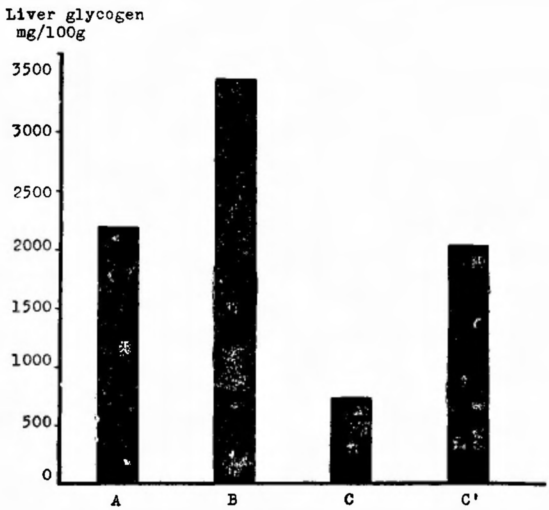
Histochemical Investigation on Liver Fat: A portion of excised liver was investigated histochemically by means of Sudan III-Hematoxylin staining.

2. Experimental Results

i) Liver Glycogen Content of 24-Hours-Fasted Rabbits Following Repeated Infusions of a Large Dose of Fat Emulsion

The results are summarized in Fig. 1. It was recognized that the liver glycogen content of the 24-hours-fasted rabbits infused with the sesame oil emulsion did not decrease but rather increased even after repeated daily infusions for 20 weeks (Fig. 1-B), in comparison with the control group (Fig. 1-A). On the other hand, in the

Fig. 1 Liver Glycogen Content of Rabbits Infused with Fat Emulsion for Prolonged Period (After 24 hours of fasting)



A : Control group

B : Group infused with 20% sesame oil emulsion, 10 cc per kg body weight, daily for 20 weeks.

C : Group infused with 20% cod liver oil emulsion, 10 cc per kg body weight, daily for 7 weeks.

C' : The C-group further infused with 20% sesame oil emulsion, 10 cc per kg body weight, daily for 2 weeks in addition.

group infused with cod liver oil emulsion, a marked decrease in glycogen was observed, after repeated daily infusions for only 7 weeks (Fig. 1-C). The latter group was further infused with the sesame oil emulsion, repeatedly for the subsequent 2 weeks and their liver glycogen content were again measured. At this time, it was found that their liver glycogen content had returned to normal (Fig. 1-C').

ii) Results of Histochemical Investigations on the Liver Fat of Rabbits Following Repeated Infusions with a Large Dose of Fat Emulsions

No pathological changes were observed histochemically in the liver tissue of rabbits which had been infused repeatedly with the sesame oil emulsion for 20 weeks. In a few cases, a small amount of sudanophilic lipid was found only in the KUPFFER's stellate cells, but not in the hepatic parenchymatous cells (Fig. 2).

On the other hand, in all rabbits, which had been infused with the cod liver oil emulsion for 7 weeks, severe fatty liver was observed without any exception. There was found a dense lipid accumulation in the hepatic parenchymatous cells, which was accompanied by a marked degree of vacuolen degeneration (Fig. 3).

However, when the latter group of rabbits was examined again after further repeated infusions with the sesame oil emulsion for the subsequent 2 weeks as described above, it was found that fatty liver was remarkably improved (Fig. 4) and, in some cases, the fatty infiltration in the hepatic parenchymatous cells had almost disappeared.

3. Discussion

Formally, it was generally believed that liver fat and liver glycogen content were inversely related; an accumulation of fat in liver always decreases the liver glycogen content and consequently damages liver function more or less. From this point of view, low fat and high carbohydrate and protein diet feeding has been considered as the best nutritional treatment especially for surgical cases in which a proper management of the liver function is one of the most important problems. Some workers, however, have pointed out that such conventional concept is not acceptable in all cases. BERNHARD examined recently the glycogenesis and lipogenesis in liver of rats which were fed on a choline deficient diet and discovered that both functions in the liver were not altered until a certain type of pathological change occurred in the hepatic cells in addition to the simple accumulation of fat in the liver. This finding proves the belief that the fat accumulation itself is not a direct cause of the decrease in liver glycogen content. Needless to say, an abundant supply of fat causes a more or less temporary increase in liver fat. But such a physiological deposition of fat in liver should be clearly distinguished from the pathological fatty liver which is always accompanied by liver glycogen depletion as well as functional damage of this organ.

Recent studies in our laboratory have clarified the fact that fat metabolism in vivo is divided into two types: direct and indirect oxidation process. From this point of view, we have established a definite formula for judging the propriety of fats as nutriment. Fatty acids such as higher saturated fatty acids, oleic acid, linoleic acid and linolenic acid are transfered, in the form of phospholipids, not only to the hepatic parenchymatous cells but also to the extrahepatic tissues to be oxidized (so-called direct oxidation). Therefore, these fatty acids are hardly ketogenic and cause no injurious effect on the liver. On the other hand, all or almost all fatty acids such as lower fatty acids, highly unsaturated fatty acids having more than 4 unsaturated bonds and unsaturated fatty acids having more than 20 carbon atoms (eicosenoic acid, docosenoic acid, etc.) are transfered to the parenchymatous cells of the liver in the form of phospholipids (so-called indirect oxidation), and only a part of them goes slowly into the extrahepatic tissues to a slight degree. Accordingly, these fatty acids are more ketogenic and impose a heavy burden on the liver. Furthermore, the highly unsaturated fatty acids are most easily auto-oxidized and produce peroxides. KANEDA, SHIMADA and KISHIMOTO have confirmed the fact that the auto-oxidation product is extremely harmful to the liver function destroying its mitochondria. Thus, the fatty acid composition is the most important factor concerning the nutritional propriety of fats.

As to the two kinds of fats used in the present experiment, cod liver oil contains large quantities of the above-mentioned unfavourable fatty acids which undergo, for the most part, so-called indirect oxidation, while the sesame oil is composed of fatty acids which undergo, for the most part, so-called direct oxidation; the former is one of the most undesirable fats and the latter is the best as nutriment. In the present experiment, a large dose of these fat emulsions were repeatedly infused intravenously into the rabbits over a long period without simultaneous use of glucose and vitamins; although a supplemental use of the latter two is regarded as indispensable for the smooth utilization of fat in vivo. The rabbit being a herbivorous animal has a poor capacity for the disposition of fat. Therefore the fat, in this experiment, was administered under extremely unfavourable conditions. The repeated infusions of sesame oil emulsion did not cause fatty liver nor decreased the liver glycogen content even under these conditions, while the administration of cod liver oil emulsion produced always fatty liver. These results confirm the belief that the cause of fatty liver is not a matter of quantity but quality of fat administered; namely, its fatty acid composition and degree of contamination with peroxides are the main factors in the production of fatty liver. The fatty liver which had been produced by the use of cod liver oil emulsion was markedly improved by the subsequent use of sesame oil emulsion. This result supports further the belief that the sesame oil has a lipotropic activity. This oil contains large quantities of the essential fatty acids, as TAN, TOBE and MAKI have shown. It is now becoming apparent that these essential fatty acids are effective for removing the cholesterol accumulated in various tissues, and some preparations of the essential fatty acids have come into clinical use for the purpose of preventing or improving atherosclerosis etc.. The essential fatty acids as well as inositol and choline are the main components of phospholipids, which play an indispensable role in the body as the constant element, and have a lipotropic activity.

From the results mentioned above, it is concluded that the previously described conventional conception of the antagonistic action between fat and carbohydrate in the liver must be corrected, and there is no reason for restricting fat in surgical cases, if the quality of fat to be administered is considered properly. It should be reemphasized that supply of such fat as sesame oil is rather favourable to liver function.

4. Conclusion and Summary

The author has made biochemical estimations of the liver glycogen content and histochemical investigations of the liver fat of rabbits which had been infused intravenously with a large dose of sesame oil emulsion and cod liver oil emulsion respectively for a long period and reached the following conclusions :

i) Repeated administrations of cod liver oil emulsion decrease the liver glycogen content and produce fatty liver.

ii) However, repeated administrations of sesame oil emulsion increase the liver glycogen content rather and do not cause fatty liver. This administrations are effective for the improvement of fatty liver produced by the use of cod liver oil emulsion.

II. EFFECTS OF DIETARY FAT ON LIVER GLYCOGEN CONTENT OF RATS

1. Experimental Methods

Experimental Animals : Male weanlings of "Wistar" rats were used. They were fed with a standard stock diet (ORIENTAL Koso K. K.) until their body weight reached about 100 g, and then divided into the following 5 groups according to the types of experimental diets :

i) Control group

ii) Group fed with a low fat and high carbohydrate diet (Abbreviated as low fat group, below.)

iii) Group fed with a 30% sesame oil containing diet (Abbreviated as sesame oil group, below.)

iv) Group fed with a 30% butter fat containing diet (Abbreviated as butter fat group, below.)

v) Group fed with a 30% cod liver oil containing diet (Abbreviated as cod liver oil group, below, and the last three groups are also jointly termed as the fat group.)

Table 1 Experimental Diets

	Low fat diet	30% fat containing diets
Wheat flour	60 g	20 g
Corn flour	20 g	20 g
Casein	20 g	20 g
Yeast	5 g	5 g
Salt mixture (McCOLLUM')	2 g	2 g
Fat[1)]	0	30 g
Vitamin mixture	...2)	...2)

1) Sesame oil-J. P. VI for sesame oil group, "SNOW" Brand Butter for butter fat group, "MEGANE" Brand Cod Liver Oil for cod liver oil group, were used respectively.

2) KRAMÁR's prescription was adopted: Biotin 15μg, Thiamine HCl 600μg, Riboflavin 750μg, Folic acid 100μg, Ca-Pantothenate 1500μg, Pyridoxine HCl 750μg, Nicotinic acid 6mg, Choline chloride 100mg, Inositol 300mg, B_{12} 0.2γ, C 33mg, K 0.4mg, A 540 i. u., α-Tocopherol 0.2mg, D_2 45 i. u.

These rats were placed in a room maintained at a constant temperature of 20°C and were fed ad libitum with the respective experimental diets for the period of two months prior to the undermentioned experiments. The respective diets are shown in Table 1.

Determination of Liver Glycogen Content: Rats were anesthetized with a intraperitoneal Nembutal injection and about 0.5 g of their liver tissue was excised. Measurement of its glycogen content was made following the procedure described in Part 1. The values shown in this paper are respective mean-values of four to five samples, unless otherwise described.

Histologic and Histochemical Investigation of Adrenal Glands: Animals were sacrificed by a head-blow and their adrenals were removed. Investigations were made by means of Sudan III, Sudan III-Hematoxylin and Hematoxylin-Eosin staining, following fixation with 10% neutral formol solution and the preparation of frozen slices.

2. Experimental Results

i) Changes in Liver Glycogen Content of Rats Following Intraperitoneal Administration of Glucose (Preparatory Experiment)

The author adopted LONGLEY's procedure for examination of the glycogenetic activity of rats. To determine the fitness of this procedure, a part of LONGLEY's experiment was re-examined using the control group as a preparatory experiment. The rats were fasted for exactly 24 hours and injected intraperitoneally with 2 cc of 10% glucose solution per 100 g of body weight. Thereafter, each set of 4 rats were

Fig. 5 Change in Liver Glycogen Content of Rats Following Intraabdominal Administration of Glucose
(2 cc of 10% glucose solution per 100g of body weight)

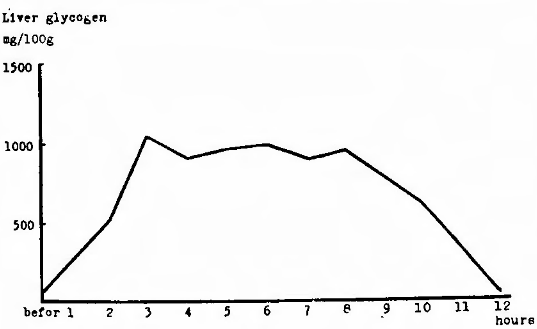
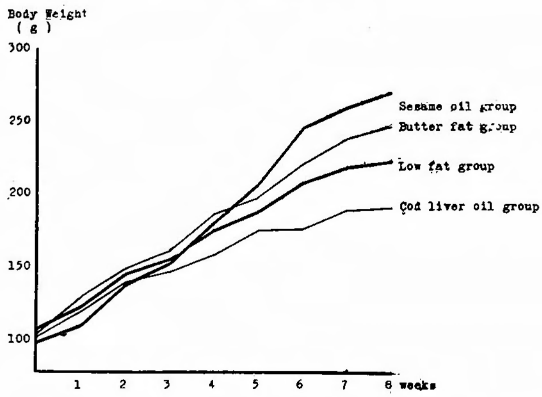


Fig. 6 Effect of Dietary Fat on Gain in Body weight of Growing Rats



successively sacrificed for the measurement of their liver glycogen content at the interval of 1 or 2 hours. As shown in Fig. 5, the result agreed with the LONGLEY's observations. It showed that the liver glycogen content reached a maximum 4 hours after the administration of glucose solution and maintained it for several hours subsequently. It indicates, therefore, that the glycogenetic activity of rats can be evaluated by measuring the 5th-hour-level of its liver glycogen, as this value represents the maximal level after the administration of glucose solution.

ii) Effects of Dietary Fats on Growth of Rats

The group-averages of the gains in body weight increased in the following order: Cod liver oil group, low fat group, butter fat group and sesame oil group,

Fig. 7 Effect of Dietary Fat on Glycogenolysis in Liver of Rat

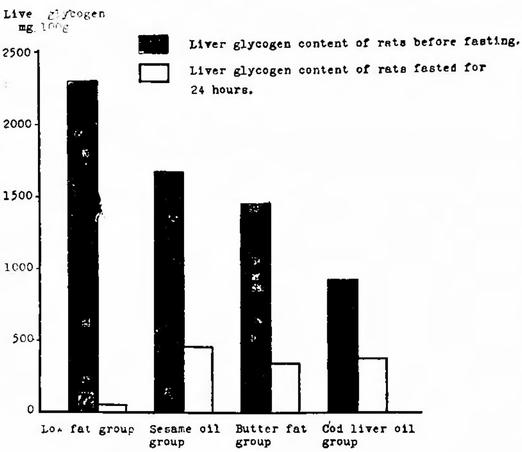
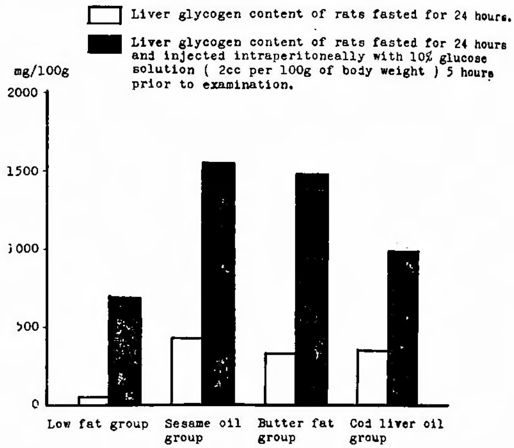


Fig. 8 Effect of Dietary Fat on Glycogenesis in Liver of Rat



as shown in Fig. 6.

iii) Effects of Previous Feeding of Fats on the Glycogenolysis of Rats during 24 Hours of Fasting

The liver glycogen content of the respective group was measured before fasting (3 hours after a renewal of the diet in each food-cup), and after 24 hours of fasting. The results are shown in Fig. 7. The low fat group, which had taken a larger amount of carbohydrates than any other group, naturally showed the highest content of liver glycogen before fasting and was followed by the sesame oil group and butter fat group showing slight difference. The cod liver oil group showed the lowest. On the other hand, the measurements after 24 hours of fasting revealed the following fact: At this time, the liver glycogen of the low fat group was almost depleted, while, in the fat groups, about 300 mg per 100 g of liver weight or more of the liver glycogen was left unconsumed. The above results proves the fact that the dietary fat, regardless of its kind, acts to delay the glycogenolysis and thus to economize liver glycogen during fasting.

iv) Effects of Previous Feeding of Fat on the Liver Glycogenesis of Rats Following the Administration of Glucose Solution

Ten percent glucose solution (2 cc per 100 g of body weight) was injected intraperitoneally into the 24 hours fasted rats of each group. The liver glycogen content of these animals was measured 5 hours after the glucose injection to evaluate the glycogenetic activity. In Fig. 8, the results are shown together with the above described values of 24-hours-fasted-levels of each group, as starting values. It is difficult to compare with quantitative accuracy the glycogenetic activity among these groups, from the above values alone, since their starting values were not uniform as described previously. It demonstrates clearly, however, at least the fact that the glycogenetic activity of both sesame oil and butter fat group are more active than those of the low fat and cod liver oil group. Between the latter two groups, there was no significant difference in increments from the starting levels of liver glycogen content, though the level of the cod liver oil group after the administration of glucose solution was higher than that of the low fat group. Accordingly, it is difficult to make a definite comparison of the glycogenetic activity of these two groups. From the above results, it is concluded that the previous feeding of fat generally accelerates the glycogenetic activity of rats, with the exception of some kinds of fats such as cod liver oil.

v) Effect of Previous Feeding of Fat on the Survival Period of Rats in Starvation

An observation on the survival periods of the low fat group and the sesame oil group was made. As shown in Table 2, there existed a remarkable difference

Table 2 Survival Time of Rat During Fasting

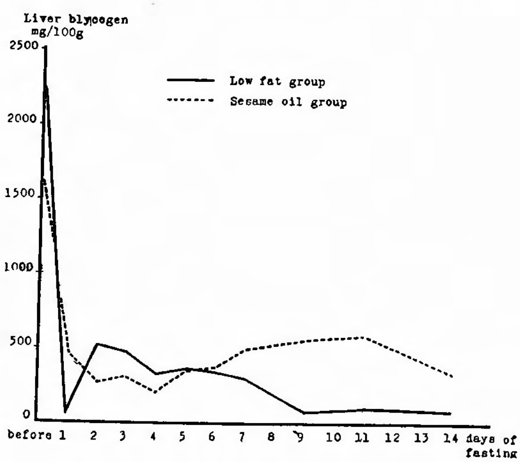
Low fat group	Sesame oil group
12 days	15 days
13 〃	18 〃
14 〃	21 〃
14 〃	21 〃
15 〃	22 〃
16 〃	25 〃
18 〃	25 〃
18 〃	
Mean 15±2.2 days	21±3.3 days

between these two groups. That is, the previous feeding of sesame oil lengthened the survival period of rats in starvation.

vi) Effect of Previous Feeding of Fat on Changes in the Liver Glycogen Content of Rat during Starvation

Rats of the sesame oil group and low fat group were maintained in a continual stage of starvation, during which 4 to 6 rats out of each group were sacrificed daily and their liver glycogen content measured. The daily changes in liver glycogen content of the above two groups during starvation are shown in Fig. 9. As shown

Fig. 9 Change in Liver Glycogen Content During Fasting



in it, both curves were observed to have in common a triphasic character. At an early stage of starvation, the glycogen content of rats was at one time lowered to the minimal level as a result of the glycogenolysis (lst phase). Subsequently, it was raised by the advance of the gluconeogenesis and this relatively high level continued for some time (2nd phase) but dropped again in the terminal stage (3rd phase). In the course of the respective phases, however, there were observed remarkable differences between these two groups, as follows. As to the low fat group, the first drop of the liver glycogen content progressed more rapidly and approached to a complete depletion by the 24 hours of fasting, as already described. Then the subsequent rise due to gluconeogenetic response started immediately, showing a clear and sharp curve from the lst to the 2nd phase. But this relatively higher level of the glycogen content did not last further than the 7th day, thereafter the 3rd phase followed. From the 9th day to death, it stayed at the minimal level. Meanwhile in the case of the sesame oil group, the first drop progressed in a markedly slower rate and the start of its subsequent rise delayed for several days, showing a gradual curve from the lst to the 2nd phase. The high level due to the gluconeogenetic response of this group was markedly prolonged as late as the 14th day of the starvation.

vii) Effects of Previous Feeding of Fat on Histologic and Histochemical Manifestations of the Adrenocortical Response to Starvation

It is a well known fact that the rate of the gluconeogenesis in the liver is not only affected by the condition of the liver function, but is also controled sensitively by the blood glucocorticoid mobilized from the adrenal glands. From this viewpoint, histologic and histochemical investigations were made on the adrenals of the above two groups on the 2nd, 7th and 14th day of the starvation period.

It is generally held that the pathologic changes which are observed in adrenal cortices of rats subjected to starvation, are of the same characters as those of rats subjected to continuous non-specific stresses, and it shows a figure of the "alarm reaction". Findings obtained in the present investigations verified the fact that there were distinctive differences in the course and intensity of the changes between the two groups as described in the following. On the 2nd day of starvation, adrenals of both groups showed a widely spread hyperemia, the lipid granules in their fasticulata zone were found to decrease moderately in density and the so-called sudanophobic zone became extremely obscure or completely disappeared (Fig. 10, Fig. 15). These changes are now regarded as a manifestation of hyperactivity of this gland. On the 7th day of starvation, the changes were observed to have become further intensified especially in the low fat group, so that a remarkable difference could be observed in the density of lipid granules in the fasticulata zone of the two groups (Fig. 11, Fig. 16). When starvation was continued to the 14th day, the differences between the two groups became more pronounced. In general, in the fasticulata zone of the low fat group at this stage, an extreme depletion of the lipid granules occurred and its cell-collumnes became irregular. Furthermore, in many cases, there were clearly observed a marked cytolysis and formations of vacuoles and coarse lipid-droplets in the fasticulata zone, which were generally believed to be due to an exhaustive change of these cells (Fig. 17, Fig. 18, Fig. 19). On the other hand, the adrenals of the sesame oil group at this stage showed only an similar intensity of change to that observed on the 7th day. There was observed an ample amount of fine lipid granules remaining in their fasticulata zone and, unlike the low fat group, no exhaustive change was found at all (Fig. 12, Fig. 13, Fig. 14).

The fasticulata zone of the adrenal cortex is now believed to be the main site for the production of glucocorticoid. Therefore, the above findings observed chiefly in this zone are considered to be significant as they are in agreement with the previously described changes in the liver glycogen content. The results of these two types of investigations lead us to the following assumption: The adrenocortical response to starvation is forced to start earlier and more actively, and its exhaustion is induced earlier, in the low fat group than in the sesame oil group.

3. Discussion

Needless to say, the animal relies upon his own stored calorigenic substances for the source of energy during fasting. ROBERTS and SAMUELS et al. have pointed out that the dietary history of animals is an important factor determing the apportionment between his storage-carbohydrate and fat which can be utilized during fasting. Namely, the previous feeding of a high carbohydrate diet accelerates the utilization of storage-carbohydrate and suppresses that of storage-fat in the subsequent fasting stage; the previous feeding of a high fat diet causes the reverse. Such phenomena are clearly demonstrated also in the results of the present experiments. In these experiments, a higher rate of glycogenolysis during fasting was observed in the low fat group of rats. It means that these animals are disposed to rely mainly on carbohydrate for their energy source. Therefore, their storage-carbohydrate is consumed more rapidly in fasting. On the other hand, the animals fed with the 30% fat containing diet have less disposition than the low fat group to rely on carbohydrate and can utilize their storage-fat more effectively from the early stage of fasting so that their storage-carbohydrate is economized. In short, there exists a marked difference between these two groups in the carbohydrate requirement; it is greater in the low fat group than the fat groups. The much more active glycogenesis observed in the fat groups is another proof of the fact that the metabolic patterns of these animals are inclined to carbohydrate assimilation. This concept may serve also to explain the results of the previous experiment (Part 1), in which, after 24 hours of fasting, the liver glycogen content of rabbits repeatedly infused with the large dose of sesame oil emulsion showed a higher level than the control group. It is concluded that a prolonged supply of fat, dietary or by parenteral infusion, adapts the animal to the fat utilization and enables it to economize the storage-carbohydrate in fasting.

The question still remains unanswered as to how such adaptative alteration is induced in the metabolic pattern. Concerning this problem SAMUELS et al. have merely postulated that some modifications of the specific enzyme systems may possibly contribute to it. Its relationship with the hormonal function should also be given consideration. Until recent times, many workers have suggested that the dietary factors may have some effects on the intrinsic hormonal balance in vivo. For instance, DUNKAN has pointed out that a high fat diet may increase the production of the pituitary hormones. Moreover, the metabolism of the steroid hormones is considered to have a close relationship with the fat metabolism, as described below. Hence, it is highly possible that an alteration of the hormonal balance may contribute to the above adaptative metabolic pattern.

Aside from its intimate mechanism, this adapting effect of fat is considered to have a great nutritional significance. The correct evaluation of this significance would be indispensable for understanding perfectly the specific nutritional effect of fat. It deserves a more detailed discussion, which shall be given below.

Protein-Sparing and Growth-Promoting Action of Fat

There have been many reports concerning the fact that fat has a growth-promoting and protein-sparing action; therefore, carbohydrates can not totally substitute for the fat. DUEL et al. have noted progressively higher gains in body weight in young rats when they were fed with diets containing increasing proportions of fat. SWANSON et al. have demonstrated the protein-sparing effect of fat with the experimental studies of the nitrogen excretion of rats. PANZER et al. have proved further that the fat deficient rats excrete a significantly larger amount of amino acids such as phenyl alanine, valine, lysine and methionine. Also in our laboratory, a number of experimental and clinical studies concerned with this problem have been performed by use of fat emulsions. OSA proved that the intravenous infusion of fat emulsion was very effective for the improvement of hypoproteinemia. KUYAMA and HANAFUSA have studied the protein metabolism of surgical patients who were treated with intravenous infusions of sesame oil emulsion and have confirmed the belief that the protein-sparing effects were also displayed in such surgical cases. Furthermore, KURATA has verified that a dietary as well as parenteral supply of fat had a favourable effect on the wound-healing of experimental animals. As mentioned previously, the growth-promoting effect of dietary sesame oil was demonstrated in the present experiment.

The physiological significance of the essential fatty acids should be given first consideration in seeking to understand the intimate mechanism of this protein-sparing action of fat. It is a well known fact that the lipid is not only stored or mobilized as a calorigenic substance in vivo, namely as the variable element, but also exists as the constant element in various organs and plays an important role in the specific function of these organs. Recent studies have further clarified the fact that the latter type of lipid contains large quantities of the essential fatty acids and exists mainly in the form of lipoprotein combined with proteins. This very lipoprotein, rather than the protein itself, has come to be regarded as the constructive element of tissue cells. In other words, the lipoprotein formation with lipid is considered to be an indispensable process in causing protein to be assimilated smoothly and in its being stabilized securely in cells and tissues. Considering these facts, a sufficient supply of fat, especially the essential fatty acids which can never be synthesized in vivo, is believed to be an indispensable factor in preventing excessive dissimilation of protein and in promoting its assimilation. HANAFUSA in our laboratory has pointed out the fact that administration of the fat emulsion extending from the preoperative to the postoperative period has more favourable effects on the postoperative protein metabolism than the administration of it limited to only the postoperative period. It is difficult to understand this finding when considered only from the view-point of the essential fatty acids. It is highly probable that the fat adapting effect as mentioned previously may play an important role in this connection.

It is generally believed that the material of the gluconeogenesis in vivo is mainly limited to protein and the synthesis of carbohydrate from fat is negligeable in practice. It means that the catabolic process of protein is simultaneously accelerated whenever the gluconeogenesis is advanced in vivo as a glucostatic mechanism compensating the insufficient carbohydrate intake. In other words, the protein is a type of substitute for carbohydrate reserves. This concept yields another explanation of the protein-sparing action of fat. As already discussed, the metabolic pattern of animals which have been fed with a diet predominant in carbohydrate, is characterized by an exceedingly high rate of the carbohydrate utilization. It is easily assumed that such a metabolic tendency relying on carbohydrate is succeeded by an exceedingly high rate of the protein catabolism during the period when these animals are subjected to fasting and their storage-carbohydrate is depleted. In this regard, the adapting effect of the prolonged fat feeding, which is effective in lessening the carbohydrate requirement of the animal, is naturally considered to serve as well for suppressing the catabolism of the protein, a carbohydrate substitute, in fasting. The HANAFUSA's above-mentioned observation suggests that such adapting effects can be produced in surgical cases by parenteral nutrition with the use of the sesame oil emulsion. Following an operation, especially of the gastrointestinal tracts, patients are forced to withstand fasting or poor nutrition in the postoperative state. An ample preoperative supply of fat can help the patients to utilize effectively their own storage-fat and thus it serves to prevent the excessive destruction of protein in the postoperative poor nutritional state.

Of course, it is possible to increase the storage-carbohydrate (glycogen), directly by an administration of a large amount of carbohydrates. However, there is a limit in the capacity of the synthesis and storage of glycogen; the surplus portion of the administered carbohydrates is rapidly converted to fat, losing its peculiarity as a carbohydrate. Hence, it is clear that the preoperative administration of a large amount of carbohydrates can hardly serve to prevent the postoperative depletion of the glycogen reserve of the patient. Moreover, in view of the above adaptative mechanism, it seems that a prolonged preoperative nutrition, predominant in carbohydrates, increases the carbohydrate requirement of the patient and accelerates the consumption of the protein as a carbohydrate-substitute in the postoperative state.

Effect of Fat on the Ability of the Animal to Resist Starvation

The results of the present experiment have coincided well with that reported by ROBERTS, SAMUELS and REINECKE. That is, the survival period of fasted rats is markedly prolonged by the previous feeding with a high fat diet. This effect of fat can be at least partially accounted for by its protein-sparing action. Our experimental results concerning the changes in the liver glycogen content and the adrenocortical function during starvation are most helpful in elucidating clearly its intimate mechanism. When discussing this problem from the aspect of the homeostatic mechanism of the organism, it seems most profitable to divide the period of starvation into the following three stages.

i) The lst stage of starvation (Transitional Stage): This stage corresponds to the period ranging from the start of the fasting to the time when the storage-carbohydrate is depleted to the minimal level. During this period, the metabolic pattern of the animal is not affected essentially by starvation and his storage-carbohydrate, fat and protein are still utilized nearly in the same manner and proportion as in the pre-fasting period. The one-sided progress of the decrease in liver glycogen due to glycogenolysis can be regarded as the most characteristic manifestation of this period. Of course, the above-mentioned glycogen-sparing action of fat prolongs this period.

ii) The 2nd stage of starvation (Stage of Reaction): The depletion of the storage-carbohydrate, if it goes beyond a certain limit, affect the organism as a stress and originates the characteristic metabolic response to starvation. This stress stimulates the pituitary-adrenocortical system to mobilize the glucocorticoid into the circulating blood, and to accelerate the gluconeogenesis in the liver. This reaction serves effectively to maintain normal carbohydrate homeostasis during starvation, but accomplishes it at the sacrifice of protein. The liver glycogen content, when once decreased to the lowest level at the end of the lst stage, rises again thereafter. This phenomenon is considered to be an expression of the above-mentioned gluconeogenetic response, which is the characteristic manifestation of the 2nd stage of starvation.

iii) The 3rd stage of starvation (Stage of Exhaustion): When starvation is further continued, the shortage of the storage fat and of so-called labile or dispensable reserve protein become threatening, the liver function is damaged increasingly and the adrenocortical reserve power is exhausted. As a result of this, it becomes more difficult to maintain the vital homeostasis of the animal. Then the latest stage of starvation is commenced and the destruction of the various organs and tissues proceeds irreversibly until death. The 3rd phase of the changes in the liver glycogen content, namely its terminal drop, may not be a direct expression of such a severe exhaustive state. However, it should be at least interpreted as one of the important signes warning the approach of this pathological stage of starvation.

Thus the triphasic fluctuation of the liver glycogen content during starvation is correctly understood from the dynamic aspect of the homeostatic function of the organism. This concept enables us to discuss in more detail the effect of fat on the survival period of the fasted animals. As has been already said, animals fed with a low fat high carbohydrate diet are disposed to depend mainly upon the carbohydrate reserve for their energy source. In other words, they are highly susceptible to the carbohydrate deficiency. Therefore, the starvation-stress affects them much more aggressively inducing the active gluconeogenetic response at an early stage. Hence, the protein catabolism of these animals is accelerated, the exhaustion of their liver and adrenocortical function is induced earlier and consequently their survival period is markedly shortened. The significantly acute fluctuation of the liver glycogen level observed in this group of animals illustrates clearly the above aspect. On the other hand, animals which are well fortified by the fat feeding can utilize most smoothly their storage-fat during starvation to protect themselves from the rapid depletion of the carbohydrate reserve. This fat-adapting effect is believed to make the starvation-stress much milder. It lessens the protein catabolism and supports well the hemeostatic function of the fasted animals. This aspect is reflected faithfully in our findings, which have shown that the gluconeogenetic response of these animals began very gradually and lasted till late in the starvation period. Moreover, the histochemical investigations have confirmed the belief that the adrenocortical reserve power of these animals is well preserved for a significantly longer period of starvation.

However, it is neccessary to consider not only such stress moderating effects as are above discussed, but also an action of the essential fatty acids, for a complete understanding of the favourable effects of fat feeding on the adrenocortical function during starvation. It must be pointed out that the content of essential fatty acids in the low fat diet used in the present experiment was assumed to be very poor, though it was not completely free of fat. Accordingly, the fact can not be ignored that the low fat group of rats suffered from latent deficiency of the essential fatty acids.

It has been generally accepted that a main precursor of all kinds of steroid hormones is the cholesterol-ester existing in the adrenal cortex. Recent studies have further confirmed the fact that the essential fatty acids have a close relationship with the cholesterol metabolism and esterification with the essential fatty acids is an indispensable process in the transfer of cholesterol from blood to the various tissues. In our laboratory, JINDO and MAKI have verified the fact that the essential fatty acids are the main component fatty acids of the cholesterol-ester in the adrenal cortex. Moreover, NAGASE have proved that essential fatty acids deficiency causes an abnormal increase in capillary permeability predisposing to edema formation, and a weakened adrenocortical function may contribute to this phenomenon. In these respects, it may reasonably be postulated that the essential fatty acids play an important role in the production of adrenal steroid hormones and its supply is indispensable in order to prevent the exhaustion of the adrenocortical reserve power of the organism subjected to stresses.

From the foregoing discussion, it is established that the specific nutritional effects of fat, aside from its caloric value, are attributed mainly to the following two factors: The action of the essential fatty acids and the fat adapting mechanism. It should be emphasized that a previous supply of fat can be expected to display the above-mentioned favourable effects in the subsequent undernutritional or stressful state. This previous supply having a functional relationship to the adrenal cortex strengthen the resisting ability of the organism against stress and makes the starvation-stress milder. This fact is considered to have an important significance in clinical case, and supports our opinion that the proper supply of fat is an indispensable procedure in accomplishing perfect preoperative nutrition, for the patient can be protected thereby from the postoperative metabolic disorders better than any other means. Until recently, it has generally been believed that an abundant administration of fat decreases the liver glycogen and damages the liver function; therefore, it is prohibited in surgical cases. But our results confirm the fact that this liver damaging effect of fat is mostly due to the indiscriminate use of fat, and the harm of fat can be completely excluded by using a properly selected fat, such as refined sesame oil. In addition, fats of the latter type have a liver protective action, preventing the depletion of the liver glycogen.

4. Summary and Conclusion

In order to clarify the nutritional significance of fat, a series of experiments were performed using rats which were fed with a low fat high carbohydrate diet and with a few kinds of diets containing fats respectively. Examinations of glycogenolysis, glycogenesis and gluconeogenesis in liver of the animals were mainly dealt. In addition to these, observations of their growth and histological and histochemical investigations of their adrenal glands were made.

Based on the general findings, the author reached the following conclusion.

1) The prolonged feeding with high fat diets produces fat adapting effect in the organism. The essential effect is primarily the acceleration of the utilization of fat and the suppression of that of carbohydrate; in certain conditions such as starvation, the suppression of the protein catabolism should be counted in.

2) Carbohydrates can not totally substitute for fat, for the latter has numerous specific nutritional actions, besides its caloric value. These specific effects of fat are attributed mainly to the following two factors: (i) the action of the essential fatty acids and (ii) the fat adapting mechanism.

3) It is well established that fat is not at all injurious to the liver function, but is rather favourable to this organ, when the quality of fat and the contamination of peroxides are properly considered.

4) We believe, therefore, that supplying the proper fat is an essential procedure for securing complete nutrition in surgical cases.

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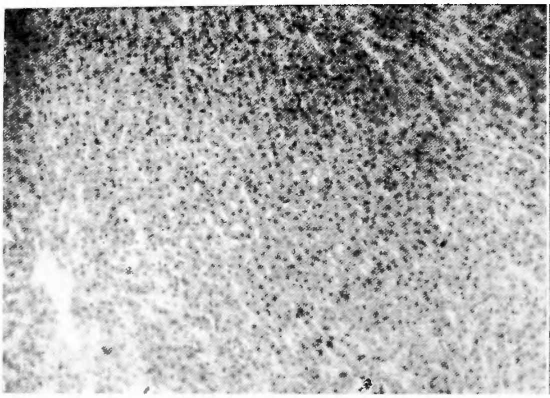
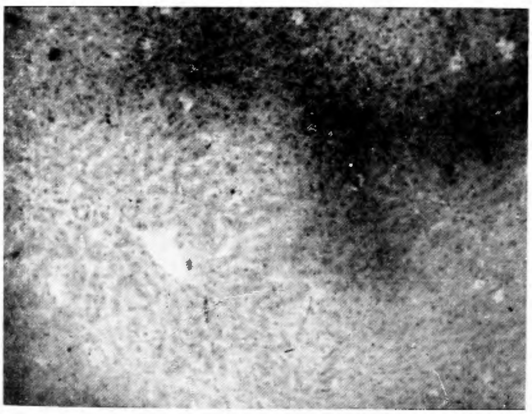


(Fig. 2)

(Fig. 3)

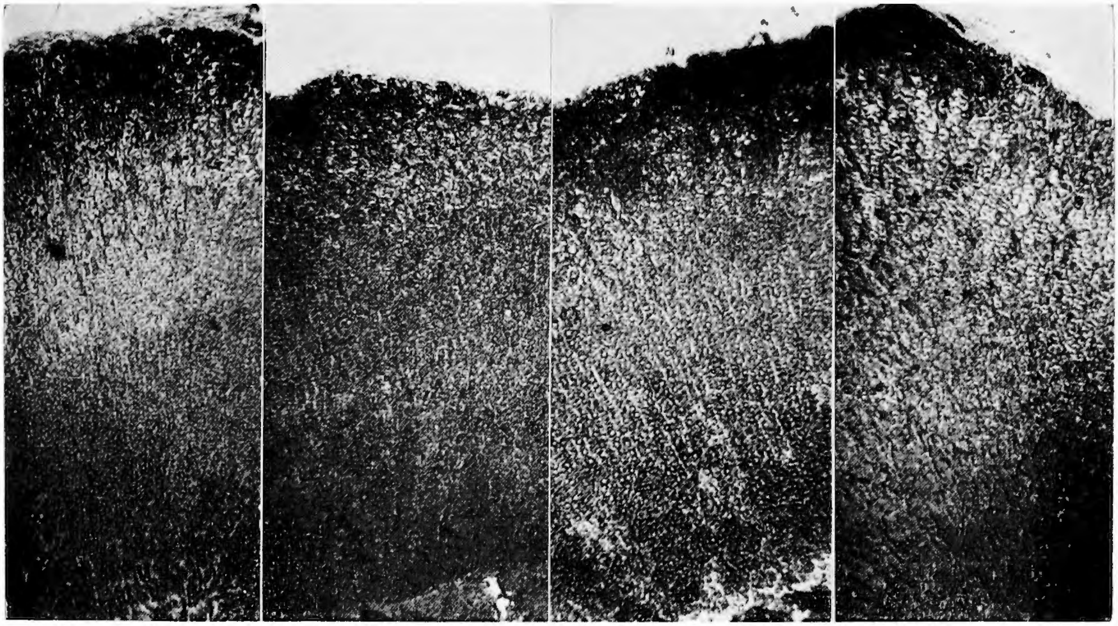
Fig. 2 Liver of rabbit infused with sesame oil emulsion daily for 20 weeks, showing no abnormal change. Sud. Ⅲ-H.

Fig. 3 Liver of a rabbit infused with cod liver oil emulsion daily for 7 weeks, showing severe fatty liver. Sud. Ⅲ-H.



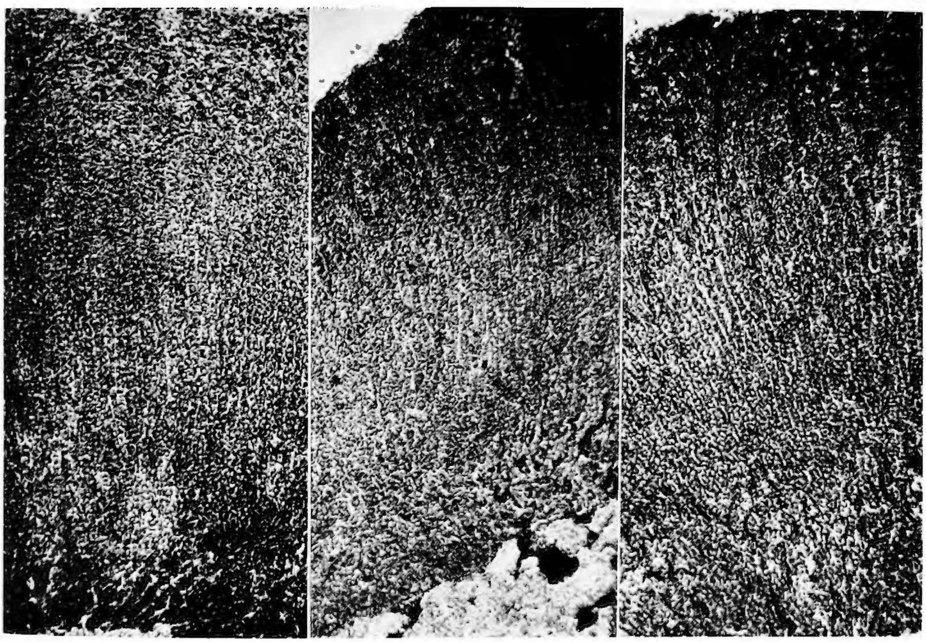
(Fig. 4)

Fig. 4 Liver of the same rabbit which is shown in Fig. 3, after additional infusions with the sesame oil emulsion daily for 2 weeks, showing marked improvement of the fatty liver. Sud. Ⅲ-H.



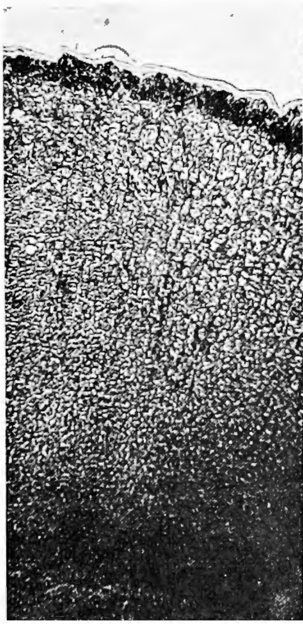
(**Fig. 10**) (**Fig. 11**) (**Fig. 15**) (**Fig. 16**)

Fig. 10 Adrenal gland of sesame oil group, after 2 days of fasting. Sud. III-H.
Fig. 11 Adrenal gland of sesame oil group, after 7 days of fasting. Sud. III-H.
Fig. 15 Adrenal gland of low fat group, after 2 days of fasting. Sud. III-H.
Fig. 16 Adrenal gland of low fat group, after 7 days of fasting. Sud. III-H.

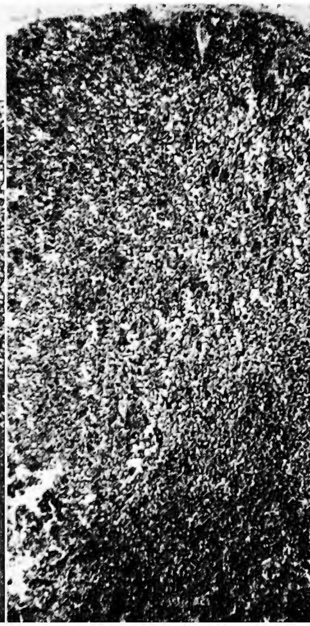


(**Fig. 12**) (**Fig. 13**) (**Fig. 14**)

Fig. 12 Adrenal gland of sesame oil group, after 14 days of fasting. Sud. III.
Fig. 13 Adrenal gland of sesame oil group, after 14 days of fasting. Sud. III-H.
Fig. 14 Adrenal gland of sesame oil group, after 14 days of fasting. Sud. III-H.



(Fig. 17)



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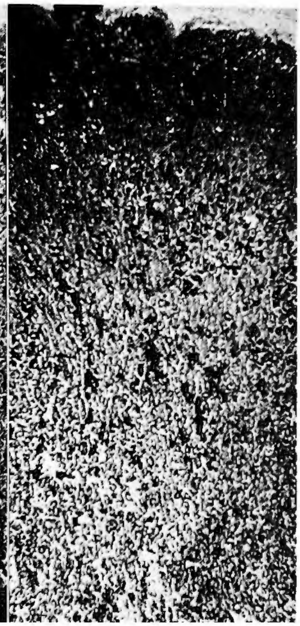

(Fig. 19)

Fig. 17 Adrenal gland of low fat group, after 14 days of fasting. Sud. Ⅲ.
Fig. 18 Adrenal gland of low fat group, after 14 days of fasting. Sud. Ⅲ-H.
Fig. 19 Adrenal gland of low fat group, after 14 days of fasting. Sud. Ⅲ-H.

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和文抄録

肝糖原量を中心としてみた脂質の栄養学的意義に関する実験的研究

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大量のゴマ油乳剤及び肝油乳剤をそれぞれ長期間連日静脈内に注入した2群の家兎について，肝糖原量ならびに脂肝発生の有無を検討した．

別に，低脂質食餌，ならびに30％の割合にゴマ油，バター脂或いは肝油を含有せしめた高脂質食餌によつてそれぞれ餌育した各群の白鼠について，肝 Glycogenolysis 及びブドー糖負荷時に於ける肝 Glycogenesis を比較検討した．

更に，低脂質食餌飼育群ならびに30％ゴマ油含有食餌飼育群の白鼠の飢餓時生存日数，及び飢餓期間中に於ける肝糖原量の変動を検討するとともに，その際に副腎皮質の示す態度を組織学的ならびに組織化学的に観察した．

そして以上の実験の結果から次の結論に達した．

1）低級脂酸，高度不飽和脂酸，エイコセン酸，ドコセン酸等生体内で所謂間接的酸化型式によつて処理される脂酸を多量に含有し，過酸化物混入の危険性も大である肝油の大量の投与は，常に肝糖原量の低下と脂肝を惹起する．

2）しかし，上記の有害脂酸を含有せず，生体内で所謂直接的酸化型式によつて処理され得る脂酸から構成され，しかも不可欠脂酸を豊富に含有するゴマ油の投与は，決して脂肝あるいは肝糖原量低下を招来しないのみならず，寧ろ抗脂肝的作用を発現し，既に惹起されている脂肝の改善を促進する．

従つて，手術前後等の患者に対して大量の脂質投与を忌避する主要な理由として挙げられていた脂質の肝障害作用なるものは，脂質投与の過量に起因するというよりは，主として投与脂質の質の撰択が正しく行われなかつたことの結果であり，適切な脂質を用うる限り，脂質投与は肝機能に対しても好影響を及ぼすと考え得る．

3）長期間の脂質投与は，脂質の利用亢進，糖質の異化抑制及びその同化亢進を本質とする脂質適応作用を生体に及ぼし，その結果，著明な貯蔵糖質節約効果を発現せしめる．

4）脂質の投与は生体に特異な蛋白節約作用を及ぼすが，この効果の発現機序には，脂質適応作用が重要な役割を果している．

5）脂質の充分な前投与は飢餓に対する生体の抵抗性を増強せしめる．これは，脂質適応作用によつて，飢餓 Stress が緩和され，飢餓時に於ける蛋白質の過剰な崩潰が防止され，副腎皮質機能を主役とする生体ホメオスタシス調節機能が有効に温存され得るからであるが，更に，不可欠脂酸を充分に補給して置くことが，副腎皮質予備能力を増強し，その疲憊を防ぐに有効であると云う可能性も，この際の要因として無視出来ないと思われる．

6）以上の見地からも，手術前相当長期間に亘つて充分な脂質を積極的に補給して置くことは，極めて合理的な準備処置であると云い得る．